STUDY ON SERUM HOMOCYSTEINE LEVEL AS A PROGNOSTIC MARKER FOR ALCOHOLIC LIVER CIRRHOSIS AND VIRAL CIRRHOSIS PATIENTS

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Certificate

This is to certify that this dissertation entitled **"STUDY ON SERUM HOMOCYSTEINE LEVEL AS A PROGNOSTIC MARKER FOR ALCOHOLIC LIVER CIRRHOSIS AND VIRAL CIRRHOSIS PATIENTS",** submitted by **Dr.P.Subramanian** to the faculty of Medical Gastroenterology, The Tamilnadu Dr.MGR Medical University, Guindy, Chennai-600032, in partial fulfillment of the requirement for the award of DM Degree, Branch IV (Medical Gastroenterology) is a bonafide work carried out by him under my direct supervision and guidance.

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Contents

Abstract

Study Objective: To study fasting serum Homocysteine level as a prognostic marker in patients with alcoholic liver cirrhosis and viral cirrhosis, comparing the homocysteine level with MELD score and Child-Turcotte-Pugh scores.

Design and Setting: Prospective study, Govt. Peripheral Hospital, Anna nagar, Chennai.

Patients: Fifty alcoholic cirrhosis and 15 viral cirrhosis patients.

Study period: Between December 2009 and April 2011.

Results: Serum homocysteine levels are seen elevated in 41 out of 50 (82%) alcoholic cirrhosis patients compared to 1 out of 15 (6.67%) viral cirrhosis patients. The serum homocysteine level in alcoholic group is 23.4 $\pm$ 7.91 μmol/L compared to 10.05 $\pm$ 2.75 μmol/L in viral cirrhosis group. The '*p*' value for variation in serum homocysteine level corresponding to both CTP and MELD scores is < 0.0001, in both alcoholic and viral cirrhosis patients.

Conclusion: Serum homocysteine levels are elevated in alcoholic cirrhosis than in viral cirrhosis and correlate well with CTP and MELD scores in alcoholic cirrhosis patients.

Keywords: Homocysteine, Cirrhosis, MELD and Child-Turcotte-Pugh score.

INTRODUCTION

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INTRODUCTION

Liver plays a central role in the synthesis and metabolism of homocysteine, given the fact that dietary methionine is predominantly metabolized in the liver. It is well known that serum homocysteine level increases in non alcoholic fatty liver disease (NAFLD) and non alcoholic steatohepatitis (NASH) patients. Recent studies have shown that serum homocysteine levels are elevated in chronic alcoholism and liver cirrhosis patients also. Homocysteine is a well known nerve and vascular endothelial toxin, promoting mortality, coronary atherosclerosis, myocardial infarction, stroke, and dementia. Recently it is found that homocysteine can induce liver diseases also, by promoting hepatic fibrogenesis.

Homocysteine metabolism requires cofactors of vitamins folate, B12 and pyridoxine, but the metabolism of these vitamins is impaired in cirrhotic patients. Homocysteine regulators called methyl donors are required to reduce serum homocysteine level. Methylation is a vital part of many biochemical processes in the body involving DNA, proteins and lipids. As improper methylation affects the brain and nervous system, correcting hyperhomocysteinemia will improve mental and emotional health as well.

Though several studies have proved hyperhomocysteinemia in alcoholic liver disease and stressed the importance of reducing the serum homocysteine level, there are only very few studies which compared the serum level of homocysteine with the severity of liver disease. This study is undertaken to compare the degree of elevation of serum homocysteine level with severity of liver disease in alcoholic and viral cirrhotics and to know whether serum homocysteine level can be used as a prognostic marker to assess the severity of liver disease.

REVIEW OF LITERATURE

Homocysteine

Homocysteine is a homologue of the amino acid cysteine, with the formula $HSCH_2CH_2CH(NH_2)CO_2H$, not obtained from the diet, instead, bio-synthesized from the essential amino acid methionine (Selhub 1999), that inflicts damage to the inner arterial lining (endothelium) by degrading the structural proteins of arteries and other cells of the body. Methionine is found abundantly in meat, seafood, dairy products and egg. Vegetables with few exceptions (sesame seeds and Brazil nuts) are low in methionine. Even such protein rich legumes as beans, peas and lentils contain relatively small amount of methionine compared to animal derived foods. Homocysteine exists in several forms and the sum of all homocysteine forms is called total homocysteine. It is biosynthesized from methionine by the removal of its terminal C8 methyl group. First, methionine receives an adenosine group from ATP, a reaction catalyzed by S-adenosyl-methionine synthetase, to give S-adenosyl methionine (SAM). SAM then transfers the methyl group to an acceptor molecule, (i.e., norepinephrine as an acceptor during epinephrine synthesis, DNA methyltransferase as an intermediate acceptor in the process of DNA methylation). The adenosine is then hydrolyzed to yield L-

homocysteine. L-Homocysteine has two primary fates: conversion via tetrahydrofolate (THF) back into L-methionine or conversion to L-cysteine (Champe et al., 2008). Homocysteine can be recycled into methionine or converted into cysteine with the aid of B-vitamins. Hyperhomocysteinemia irrespective of its cause is an independent risk factor for stroke, coronary and peripheral arterial diseases (Russo et al., 2004). The effects of Homocysteine on collagen also impact the protein matrix of bone. It is converted to either L-methionine or L-cysteine. This occurs in a multi step process requiring certain B-vitamins. The B-vitamins (N5-methyl tetrahydrofolate, and B12) and the enzyme 5,10-methylene tetrahydrofolate reductase are needed to complete this process. When the B-vitamins folic acid, pyridoxine and cyanocobalamine are lacking in the system, the homocysteine cannot be converted properly, which causes high homocysteine levels to build up in the body (Millet et al., 1994).

Many clinical testing laboratories consider a homocysteine value between 5 and 15 micromol / litre as healthy (Ueland PM, et al; 1993). However, studies indicate that adults with homocysteine values more than 6.3 micromol per litre are at increased risk of atherosclerosis, heart attack and stroke. After age 50, a more practical value for homocysteine is 7 to 8 micromol per litre. Life

extension foundation also recommends the target values for homocysteine as 7 to 8 micromol per litre.

Raised homocysteine levels have also been linked to

- Stroke
- Vascular Disease
- Liver Disease
- Kidney Disease
- Thyroid diseases
- Alzheimer’s disease and dementia
- Depression
- Erectile dysfunction
- Eye disease
- Hearing loss

Causes of hyperhomocysteinemia

- Increasing age
- Consuming a lot of coffee or alcohol
- Smoking
- Eating large amounts of red meat or chicken

- Gene expression – MTHFR Gene or “poor methylation gene”
- Levadopa - medication for Parkinson’s Disease
- Antiepileptic medications - used for preventing seizures
- Niacin (B-vitamin) - used for lowering cholesterol
- Methotrexate - used for treating inflammatory diseases
- Fenofibrate - used for lowering cholesterol
- Metformin - used for treating diabetes ((Desouza, et al; 2002)
- Nitrous oxide – used in anaesthesia
- Pyrimethamine – antihelminthic
- Sulfasalazine – used in inflammatory bowel disease
- Intense prolonged physical exercise (Van Meurs et al., 2004)
- Diabetes
- Renal failure
- Hypothyroidism
- Malabsorptive conditions
- Inflammatory bowel disease

Methods of lowering homocysteine level

Dietary intake of folate, vitamin B12 and vitamin B6 are the chief nutritional determinants of blood homocysteine concentrations, with folate being the

predominant vitamin. N5-formyl tetrahydrofolate is the important methyl donor in our body. Methylation is a vital part of many biochemical processes in the body. Methylation becomes impaired, as we get older. Supplementation with B- vitamins folic acid, pyridoxine and cyanocobalamine will reduce the serum homocysteine level (Van Guldener and Stehouwer 2001: Melinda 2006). Following are the methods to be adopted for lowering serum homocysteine level:

- Reduce homocysteine accumulation
- Increase homocysteine "re-methylation"
- Routine blood tests to check homocysteine levels
- Screening to test for genetic defects that cause high levels
- Addressing symptoms associated with elevated homocysteine such as:
    - High blood pressure
    - Diabetes
    - Coronary artery disease
    - Low thyroid function

Serum homocysteine level can be reduced by avoiding methionine rich diet, particularly the non vegetarian food items, avoiding smoking, coffee intake and alcohol, doing regular physical exercise and weight reducing.

Alternatively homocysteine reduction can be achieved by intake of 5-methyl tetrahydrofolate, natural folate or folic acid tablets, N-Acetyl cysteine 600 to 1800 mg daily, SAMe (S-Adenosyl methionine) 400 mg two to four times daily, taurine 1000 to 3000 mg daily, trimethyl glycine 2000 to 6000 mg daily, vitamin B12 (Cobalamin) 1 to 2 mg daily, vitamin B2 (Riboflavin) 10 to 100 mg daily, vitamin B6 (as pyridoxal phosphate) 100 to 200 mg daily, zinc 30 to 60 mg daily, micronized creatine 500 mg four to eight times daily, omega-3- polyunsaturated fatty acid rich fish oil or Choline 250 to 500 mg daily.

Methionine metabolism

Nearly one half of the daily intake of methionine is metabolized in the liver. Methionine is first metabolized to S-Adenosyl methionine (SAM) catalysed by the enzyme methionine adenosyl transferase MAT1A in the liver and in other tissues by MAT2A. S-Adenosyl methionine (SAM) functions as the principal biological methyl donor through several transmethylation pathways, as the precursor of aminopropyl groups used in polyamine biosynthesis and as a precursor of glutathione through its conversion to cysteine along the transsulfuration pathway. Under normal conditions, most of the generated

SAM is used in transmethylations reactions through methyl transferases, in which methyl groups are donated to al large number of molecules including DNA, RNA, biogenic amines, phospholipids, histones and other proteins. Methylation of these compounds may modulate cellular functions and integrity. In this process, the S-Adenosyl methionine is converted to S-Adenosyl homocysteine (SAH), which is a potent competitive inhibitor of most methyl transferases. Both an increase in SAH and a decrease in SAM / SAH ratio are known to inhibit transmethylation reactions(Purohit et al., 2007).

S-Adenosyl methionine also decreased lipopolysaccharide(LPS) stimulated tumor necrosis factor (TNF) release and increases interleukin-10 (IL-10) release in a monocyte cell line, supporting the concept that SAM may have direct hepatoprotective function and may modify LPS stimulated cytokine production.

In the kidney and liver, homocysteine is remethylated by the enzyme betaine homocysteine methyltransferase which transfers a methyl group to homocysteine via the demethylation of betaine to dimethylglycine. Folic acid can also play a critical role in the regeneration of homocysteine to methionine by means of 5-methyltetrahydrofolate(5-MTHF). Fatty liver develops in mice

that lack the gene for MTHF reductase supporting the role of this critical pathway in the development of hepatic steatosis and steatohepatitis. The transulfuration pathway requires the enzyme cystathionine synthase and vitamin B6. Once formed from cystathionine, cysteine can be utilized in protein synthesis and glutathione production. Alterations in plasma homocysteine and liver SAM and SAH contents in precirrhotic stages and in secondary biliary cirrhosis have been demonstrated in experimental animals (Mohammed R et al., 2005).

NAFLD

The prevalence of NAFLD varies from 10 to 24% in the general population, thus making it the most common liver disease in the world. Fatty liver has been documented in 8 to 10% of normal population and 70 to 80% of obese individuals. More than 20% of potential liver donors have NAFLD. Correspondingly NASH occurs in 3% of general population and 20% of morbidly obese individuals. Non alcoholic fatty liver disease which was characterized by the association of fatty liver and lobular hepatitis and chronically elevated plasma levels of alanine aminotransferase (ALT) in patients with negligible intake or absence of alcohol was first identified by

Ludwig et al in 1980. Insulin sensitivity of adipocytes depends on profile of adipokines- TNF-ά, leptin, adipokinin, plasminogen activator inhibitor-1, sex hormone, fatty acids, resistin and cortisol. Fundamental defect in NAFLD seem to be the insulin resistance that leads to impairment in insulin mediated suppression of lipolysis. Net result is increased release of free fatty acids from the adipose tissue. Increased uptake of fatty acids by hepatocytes leads to mitochondrial β oxidation overload, with consequent accumulation of fatty acids within hepatocytes. If degree of free fatty acid delivery and reesterification to triglyceride overwhelms ability to form and export VLDL, triglycerides accumulate in the liver. FFA decreases insulin mediated glucose disposal in skeletal muscle and also in liver, it induces gluconeogenesis pathways and inhibits glucose utilization. This leads to increased peripheral glucose level, which in turn leads to hyperinsulinemia. Hyperinsulinemia increases the synthesis of fatty acids in hepatocytes by increasing glycolysis and increases accumulation of triglyceride by decreasing hepatic production of apolipoprotein B-100. Thus insulin resistance leads to accumulation of fat in hepatocytes by two mechanisms: peripheral lipolysis and hyperinsulinemia. Related to this hypothesis, obesity which gives rise to insulin resistance, hypertriglyceridemia and leptin resistance are thought to play an important role (Harrison, et al., 2002). The final common pathway for development of

steatohepatitis is oxidative stress within hepatocytes. The redox state is determined by balance between pro-oxidant and antioxidant processes. Oxidative stress results from generation of reactive oxygen species and deficiency of antioxidant defenses. Multiple metabolic pathways like mitochondrial, peroxisomal, cytochrome P-450, NADPH oxidase, cyclooxygenase and lipooxygenase pathways, lead to oxidative stress. Mitochondrial reactive oxygen species deplete hepatic antioxidants and allow accumulation of more reactive oxygen species. Depletion of antioxidants like paraoxonase – 1 may enhance hepatic damage in steatosis. The Kupffer cell dysfunction induced by insulin resistance leads to increased phagocytic activity, decreased anti-inflammatory IL-10, thus leading to increased necroinflammatory activity. Also liver regeneration is impaired in fatty hepatocytes. Stellate cell activation occurs via noncytokine pathways or via cytokine pathways (TNF-ά). TNF-ά stimulates secretion of profibrogenic cytokines like IL-6, TGF-β and PDGF. Leptin secreted by activated stellate cells further leads to TGF-β production. NAFLD has the potential to progress to fibrosis, cirrhosis, terminal liver failure and hepatocellular carcinoma (Sheth, et al., 1997).

Hyperhomocysteinemia has been found in patients with type 1 and type 2 diabetes mellitus associated with premature atherosclerosis (Hoffmann MA, Hoogeveen EK, et al., 1998). Several observations suggest that there might be some links between insulin resistance and hyperhomocysteinemia (Sanchez-Margalet V, Meigs JB, et al., 2002). Homocysteine is an atherogenic and thrombogenic risk factor and may be involved in hepatic fibrosis (Torres L, et al., 1999). In a large study conducted at Turkey in 2007, serum homocysteine levels were significantly elevated (13.44 $\pm$ 3.1 μmol/L) in NAFLD patients when compared to healthy controls (8.62 $\pm$ 1.34 μmol/L) with *p* value of 0.015 (Ali cetinkaya, et al., 2007).

Cirrhosis of liver

The word "cirrhosis" derives from Greek κιρρός [*kirrhós*] meaning *yellowish, tawny* (the orange-yellow colour of the diseased liver) + English medical suffix *-osis*. While the clinical entity was known before, it was René Laennec who gave it the name "cirrhosis" in his 1819. Cirrhosis is a consequence of chronic liver disease characterized by replacement of liver tissue by fibrosis, scar tissue and regenerative nodules leading to loss of liver function. Cirrhosis is most commonly caused by alcoholism, hepatitis B and C, metabolic

diseases and fatty liver disease, but has many other possible causes. Cirrhosis of liver is associated with several metabolic disturbances in our body. Ascites is the most common complication of cirrhosis, and is associated with a poor quality of life, increased risk of infection, and a poor long-term outcome. Other potentially life-threatening complications are portal hypertension, bleeding from esophageal varices, hepatic encephalopathy, hepatorenal syndrome, hepatopulmonary syndrome and hepatocellular carcinoma. Cirrhosis is generally irreversible, and treatment usually focuses on preventing progression and complications. In advanced stages of cirrhosis the only option is a liver transplant.

Pathogenesis of alcoholic liver disease

One of the early targets of ethanol toxicity is mitochondrial fatty acid oxidation. Alcohol metabolism alters the NADH/NAD redox potential in the liver, which in turn impairs the β-oxidation and tricarboxylic acid cycle activity. Chronic alcohol consumption can increase fatty acid synthesis in humans. Gut derived endotoxin, including toxic lipopolysaccharide may trigger both cytokine release and oxidative stress. In the liver, endotoxin activates Kupffer cells, which play a major role in liver inflammation by

releasing reactive oxygen species and cytokines. Patients with alcoholic liver disease have increased levels of proinflammatory cytokines IL-1, IL-6 and TNF-ά as well as the chemokinesIL-8 and other cytokines.

Alcohol induces oxidative stress in the liver by either enhancing the production of reactive oxygen species and / or decreasing the level of endogenous antioxidants. The sources of reactive oxygen species include mitochondria, cytochrome P-450 2E1 in hepatocytes, NADPH oxidase in inflammatory cells and activated Kupffer cells.

Acetaldehyde is the most important metabolite of ethanol leading to liver damage. The toxicity of acetaldehyde is due to its capacity to form adducts with intracellular proteins which can trigger an abnormal immune response characterized by the production of antibodies directed against acetaldehyde epitopes. Adiponectin and TNF-ά suppress each other's action in their target tissues. Chronic ethanol intake decreases circulating concentrations of adiponectin and reduced adiponectin expression. Transforming growth factor-β is a key profibrotic cytokine. Acetaldehyde stimulates the production of several extracellular matrix proteins including type 1 collagen and enhances the expression of TGF-β1 in hepatic stellate cells and fibroblasts.

Pathogenesis of viral cirrhosis in HBV

Hepatitis B virus is generally not a cytopathic virus, and the severity of HBV associated liver disease is considered to be related to the intensity of the host immunologic response to the virus. The cellular immune response appears to be the arm principally involved in the pathogenesis of disease. The immunologic response to HBV encompasses both an innate or nonspecific response (for example, natural killer cells and interferons) and an adaptive immune response, including antibodies to viral antigens, human leukocyte antigen (HLA) class II restricted CD4+ T cells, and HLA class I restricted CD8+ CTLs. Induction of the antigen specific T cell response is thought to occur in lymphoid organs and results in the maturation and expansion of T cells that are specific for these viral epitopes and is followed by their migration to the liver, where they perform their effector function.

During acute HBV infection, most HBV DNA molecules are cleared rapidly from the liver via noncytopathic mechanisms mediated by cytokines that are released initially by cells of the innate immune system and later by liver infiltrating HBV specific CD8+ cells. Cell mediated immune responses are efficient in self limited infection because the responses are vigorous, multispecific and oriented toward type 1 helper (Th1) cells. Persons with

chronic HBV infection, by contrast, exhibit infrequent, narrowly focused and weak HBV specific T cell responses. In chronic hepatitis B, the mononuclear cells in liver infiltrates of patients with chronic hepatitis B at any given time are non antigen specific.

CD8+ CTLs are thought to contribute to the disease process in the liver and result in apoptosis of infected hepatocytes. To be recognized by the CD8+ CTLs, targeted hepatocytes must present viral epitopes as short peptides that have been endogenously processed and fit within the peptide-binding groove of the class I major histocompatiblity complex (MHC) molecules. The binding of the CTL T cell receptor (TCR) to the peptide-MHC complex on the hepatocyte surface can then result in the direct killing of the infected cell and release of potent antiviral cytokines by the activated CTL. Recognition by MHC class II restricted CD4+ helper T cells requires the appropriate presentation of viral peptides in the context of class II MHC molecules. The CD4+ cells produce antiviral cytokines and provide help in neutralizing antibody production.

Pathogenesis of viral cirrhosis in HCV

In chronically infected patients, the pathogenesis of liver damage is largely immune mediated. In a small subset of immunocompromised HCV infected patients among both HIV infected patients and organ transplant recipients, however, a syndrome termed fibrosing cholestatic hepatitis develops. Such cases are thought to result from direct viral hepatotoxicity of infected cells, because viral levels are typically greater than 30 million copies / mL and hepatocytes contain enormous concentrations of virus and viral proteins.

HCV infection elicits an immune response in the host that involves both an initial innate response as well as a subsequent adaptive response. The innate response is the first line of defense against the virus and includes several arms such as natural killer cell activation and cellular antiviral mechanisms triggered by pathogen associated molecular patterns (PAMPs) recognized by the cell. These processes can lead to apoptosis of infected cells within the first few hours of infection. Natural killer cells, as the effector cells of the innate immune system, also produce tumor necrosis factor (TNF-β) and interferon-ά, cytokines that are critical for dendritic cell maturation and subsequent induction of adaptive immunity. After this, however, the virus initiates a

number of mechanisms that undermine the ability of the host to control the infection.

Virus related disruption of the innate and later adaptive immune responses occur at several levels. NK cell function is slowed possibly because NK-cell mediated cytotoxicity and production of cytokines are interrupted when the HCV E2 protein binds its cellular receptor CD81. HCV - NS5A and E2 both can disrupt protein kinase R (PKR) function to suppress translation, thereby allowing viral replication to continue. In addition, NS5A inhibits 2'-5'-oligoadenylate synthetase. The ability of HCV to impair the innate immune response prevents development of a vigorous adaptive immune response to the infection. NK cells do not adequately activate dentritic cells, and as a result, the priming of CD8+ and CD4+ T cells in HCV infected patients is inadequate.

HCV specific T cells are enriched at the site of viral replication, with an increased number in the liver when compared with the peripheral blood. CD8+ lymphocytes predominate, suggesting that cytotoxic T lymphocytes are the main perpetrators of hepato cellular injury. The T cell immune response in the liver may result in direct lysis of infected cells and inhibition of viral

replication by secreted antiviral cytokines. Antibodies to viral proteins are produced in low levels and do not appear to correlate with the stage of infection or immune reactivity. In summary, viral products play an integral role in the immune regulation that leads to chronic infection instead of viral clearance. Both the virus and the immune response probably play a role in the development of hepatocellular injury.

Methionine metabolism in alcoholic liver disease

Chronic administration of ethanol in experimental animals increases levels of plasma homocysteine, reduces liver S-Adenosyl methionine and increases S-Adenosyl homocysteine. Acetaldehyde induced inhibition of methionine synthase activity is associated with increased activity of betaine homocysteine methyl transferase, which uses betaine as a substrate to methylate homocysteine to methionine and dimethyl glycine. Reactive oxygen species generated by ethanol metabolism impair the expression of methionine adenosyl transferase with consequent reduced levels of S-Adenosyl methionine (Purohit et al., 2007). Since SAM is a precursor of glutathione, deficiency of SAM will result in glutathione deficiency which is observed in many forms of liver disease. Hepatic deficiency of MAT may be caused in

part by glutathione deficiency as glutathione is required for optimal expression of MAT activity in liver. Also hepatic MAT is sensitive to oxidative stress, and the subnormal hepatic MAT activity in patients with alcoholic liver disease could result from oxidation of MAT. Depletion of mitochondrial glutathione is thought to be one pathogenic factor in the development of alcoholic liver disease and S-Adenosyl methionine prevents depletion of glutathione in mitochondriae in experimental alcoholic liver disease. In alcoholic liver disease, serum SAM, the universal substrate for methyl transferase reactions is decreased, while levels of S-Adenosyl homocysteine (SAH), an inhibitor of methylation are decreased, and serum homocysteine are elevated. SAH is also a substrate for the bidirectional enzyme SAH hydrolase that can both regenerate homocysteine or can enhance levels of S-Adenosyl homocysteine when homocysteine level is elevated (Kharbanda KK, et al; 2009). Homocysteine is mainly reduced through the transsulfuration pathway that includes two vitamin B6 dependant enzymes, cystathionine-β-synthase and γ- cystathionase, ultimately producing the antioxidant glutathione. Since S-Adenosyl methionine facilitates the cystathionine-β-synthase reaction, reduced liver SAM levels are associated with decreased production of glutathione. Reduced SAM and elevated SAH predictably reduce the potential for methylation reactions, potentially

contributing to the activation of many genes relevant to liver injury (Esfandiari F, et al; 2010). Ethanol-induced increases in hepatic SAH also potentiate pro-inflammatory cytokine TNF-ά and cell death in ethanol-fed mice (Song Z, et al; 2004). Homocysteine is postulated to play a role in the pathogenesis of fatty liver and in experimental animals the reduction of homocysteine level by administration of betaine results in attenuating the severity of alcoholic liver disease. Deficiency of B vitamins B6, B12 and folic acid in alcoholics also lead to impaired metabolism of methionine and impaired regeneration of homocysteine and finally fatty changes in the liver. The correlation between serum cystathionine level with the severity of liver fibrosis point to the importance of the homocysteine transsulfuration pathway in alcoholic liver disease and may have important diagnostic and therapeutic implications (Valentina Medici, et al; 2010). In ethanol-fed micro pigs, folate deficiency amplified abnormal hepatic methionine metabolism, increased oxidative stress and DNA damage and promoted early development of alcoholic liver damage. In smooth muscle cells and in liver stellate cells, homocysteine impairs extra cellular matrix breakdown, by inducing the expression and synthesis of the tissue inhibitor of metalloproteinases-1 (TIMP-1) and alpha 1-procollagen (Garcia-Tevijano ER, et al; 2001). Alcohol-induced hyperhomocysteinemia in mice, is associated with

endoplasmic reticulum stress, leading to the activation of apoptotic and fat synthetic gene expression in the liver, that contribute to some of the pathologic features of alcoholic liver disease. The correction of hyperhomocysteinemia by betaine reverts these changes (Ji C, et al; 2003).

Effect of folate level on Homocysteine

Folate deficiency disturbs hepatic methionine metabolism and causes impaired catabolism of homocysteine in the liver and other tissues, decreasing cystathionine, glutathione and SAM synthesis, inhibiting homocysteine remethylation, increasing SAH and reducing hepatic choline and its precursors that could lead to liver damage (Pajares, et al; 1992). Requirements of folate are increased in obese NAFLD patients in whom the methionine cycle fails even in early stages of liver injury. Serum folate increases in these patients as a protective mechanism. Serum folate levels are inversely proportional to the body mass index in NAFLD patients and there is a positive correlation between serum total homocysteine levels and fat mass (Sandra Hirsch, et al; 2002). Folate supplementation in pharmacological doses (5mg daily) reduce the level of serum homocysteine significantly and increases the serum total antioxidant capacity, thereby reduces the risk of cardiovascular

diseases in adults with hypercholesterolemia than when compared with lovastatin (Shidfar F, et al; 2002).

Homocysteine in liver transplant recipients

Liver transplant recipients have an increased risk for cardiovascular disease because of a high incidence of obesity, arterial hypertension, diabetes mellitus, hyperlipidemia and hyperhomocysteinemia. Hyperhomocystinemia is associated with increased serum creatinine levels and renal dysfunction, and it is a frequent finding in liver transplant recipients. Treatment with folic acid 10 mg/day in liver transplant recipients, may reduce fasting serum total homocysteine levels (Herroro JI, et al; 2000). Treatment with the folic acid metabolite L-5-methyl tetrahydrofolate in a dose of 1 mg per day, effectively reduces the serum total homocysteine level in orthoptic liver transplantation recipients (Akoglu B, et al; 2008).

Homocysteine in cirrhosis

In cirrhosis patients multiple metabolic abnormalities have been detected, including higher level of serum homocysteine, lower level of serum folate, lower level of vitamin B12, increase in serum copper and decrease in zinc

levels. A significant increase in endothelial markers are also seen in chronic liver disease, like thrombomodulin, fibrinogen and von Willibrand factor, while a fall in the level of vitamin K dependant factors (as prothrombin complex, protein C, protein S) and factors synthesized in liver antithrombin are seen in cirrhosis. Hyperhomocysteinemia, a known atherogenic and thrombogenic risk factor not only increases cardiovascular morbidity and mortality, but also induces hepatic fibrosis and cirrhosis (Halifeoglu I, et al; 2004). It has been suggested that the major metabolic block in the methionine catabolic pathway in cirrhotics exists at the level of the enzyme S-adenosylmethionine synthetase because in previous studies using conventional amino-acid analyzers, no intermediates of transmethylation/transsulfuration were found to accumulate in plasma downstream of S-adenosylmethionine synthesis. Recent studies provide indirect evidence for two hitherto unrecognized alterations of methionine metabolism in cirrhotics, i.e. impairment of the transsulfuration of homocysteine at the level of cystathionine degradation and a shift in remethylation of homocysteine towards the betaine-homocysteine-methyltransferase reaction. The serum levels of methionine, N,N-dimethylglycine, N-methylglycine, cystathionine, and homocysteine were significantly higher in patients at Child stages B/C cirrhosis (Look MP, et al;

2000). Higher levels of serum homocysteine are more prominently seen in alcoholic cirrhosis patients, while increase in serum homocysteine levels is also present in non alcoholic cirrhosis patients (Bosy-Westphal A, et al., 2003).

Assessing severity of cirrhosis

To understand the expected lifespan, perioperative mortality and fitness for liver transplantation, scoring systems have been developed to assess the severity of cirrhosis. Child-Turcotte-Pugh score, MELD score and MELD-Na score are the most commonly accepted scores to asses the severity of cirrhosis.

Child-Turcotte-Pugh score

The Child-Turcotte-Pugh (CTP) score, originally developed for the assessment of the outcome of patients with cirrhosis and portal hypertension, was extended for general prognosis, and to stratify patients on the waiting list for liver transplantation. Five variables serum bilirubin, serum albumin, Prothrombin time elevation or INR, ascites and encephalopathy are

considered, and a score of between 1 and 3 is accordingly assigned to each of these factors depending on the severity as given in the table below.

Measure	**1 Point**	**2 Points**	**3 Points**
Total bilirubin, μmol/l, (mg/dl)	<34, (<2)	34-50, (2-3)	>50, (>3)
Serum albumin, g%	>3.5	2.8-3.5	<2.8
INR	<1.7	1.71-2.20	> 2.20
Ascites	None	Mild	Severe
Hepatic encephalopathy	None	Grade I-II	Grade III-IV

Some older reference works substitute PT prolongation for INR. The sum of the scores provides the Child-Turcotte-Pugh score, which corresponds to a Child-Turcotte-Pugh grade of A, B or C (Child CG, et al; 1964). CTP grade A includes patients with scores of 5 and 6, CTP grade B includes patients with scores 7,8 and 9 while CTP grade C comprises patients with scores of 10 to 15. This grading is used as a general means to verify the prognosis of the patient. For example, it can be used to determine the risk to a patient with regard to possible surgery, and also, to suggest the perceived survival of the patient over a period of time. Pharmaceutical manufacturers may use the Child-Pugh grade to suggest dose reductions, or to contraindicate the use of the drug, dependent on the degree of dysfunction of the cirrhotic liver. The use of CTP in prioritizing potential liver transplant recipients is limited by

several factors: the variables, ascites and encephalopathy, are all subjective and are influenced by medical therapy. The lack of an assessment of renal function, which is a reliable prognostic marker in cirrhosis, is an additional limiting factor.

In primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC), the bilirubin references are changed to reflect the fact that these diseases feature high conjugated bilirubin levels. The upper limit for 1 point is 68 μmol/l (4 mg/dl) and the upper limit for 2 points is 170 μmol/l (10 mg/dl). The survival rates in each CTP grades without liver transplantation are as follows.

Points	**Grade**	**One year survival**	**Two year survival**
5-6	A	100%	85%
7-9	B	81%	57%
10-15	C	45%	35%

MELD Score

MELD was originally developed at the Mayo Clinic, and at that point was called the "Mayo End-stage Liver Disease" score. It was derived in a series of patients undergoing TIPS procedures. The original version also included a

variable based on the underlying etiology of the liver disease (Wiesner RH, Malinchoc M, et al; 2000). A modification of this score was developed to predict mortality in patients with cirrhosis of different etiologies and severities of liver disease (Kamath PS, et al; 2001). This MELD score was found to be superior to the CTP score in predicting 3-months mortality and therefore the MELD score was implemented in 2002 in the United States for the prioritization of liver transplantation recipients.

The MELD score has the advantage that it is based on a multivariable analysis of objective tests for serum bilirubin, INR and serum creatinine. Compared to CTP score, it also includes assessment of renal function, another major marker of the severity of the disease. Though serum bilirubin, creatinine, and INR are usually considered objective and therefore highly reliable, they may also be influenced by therapeutic manipulations, not only by disease progression. So, one important advantage of MELD, namely the independence of the subjective judgment by a clinician, is counterbalanced in part by arbitrary laboratory values.

MELD uses the patient's values for serum bilirubin, serum creatinine, and the international normalized ratio for prothrombin time (INR) to predict survival. It is calculated according to the following formula:

$$MELD = 3.78[Log_e \text{ serum bilirubin (mg/dL)}] + 11.2[Log_e \text{ INR}] + 9.57[Log_e \text{ serum creatinine (mg/dL)}] + 6.43$$

If the patient has been dialyzed twice within the last 7 days, then the value for serum creatinine used should be 4.0. Any value less than one is given a value of 1 (i.e. if bilirubin is 0.8, a value of 1.0 is used) to prevent the occurrence of scores below 0 (the natural logarithm of 1 is 0, and any value below 1 would yield a negative result). MELD score represents a continuous variable ranging from 6 to 40.

MELD score also proved to be a reliable marker of 1-year and 5-year survival across a broad spectrum of liver diseases including alcoholic cirrhosis and alcoholic hepatitis. In addition, MELD score has been shown to be a good prognostic marker in cases of variceal bleeding, spontaneous bacterial peritonitis, and hepatorenal syndrome (Chalasani N, et al; 2002). Independent of the cause of cirrhosis, high MELD score was shown to be associated with a decrease in residual liver function as measured by monoethylglycinexylidine test.

In interpreting the MELD Score in hospitalized patients, the three month mortality is as follows:

- MELD score ≥ 40 — 71.3% mortality
- MELD score 30–39 — 52.6% mortality
- MELD score 20–29 — 19.6% mortality
- MELD score 10–19 — 6.0% mortality
- MELD score <9 — 1.9% mortality

Lab Test Frequency

MELD score greater than or equal to 25; Lab testing needed every 7 days.

MELD score 24-19; Lab testing needed every 30 days.

MELD score 18-11; Lab testing needed every 90 days.

MELD score less than or equal to 10; Lab testing needed every year.

There are four Special Case Exceptions that will be assigned a higher MELD score than that assigned by the patient's laboratory test results - Hepatocellular Carcinoma, Hepatopulmonary Syndrome, Primary Oxaluria and Familial Amyloidosis.

MELD – Na

It was felt that patients with refractory ascites, normal creatinine, and preserved hepatic function could be under-scored with MELD. In particular, it was shown that persistent ascites and low serum sodium identified a subset of patients with relatively low MELD score (below 21) and a high risk of early death. During cirrhosis, hyponatremia results from solute-free water retention. Systemic arterial vasodilation leads to the release of antidiuretic hormone which, in turn, induces dilution hyponatremia. The activation of these mechanisms correlates with the degree of portal hypertension.

Several studies have shown that hyponatremia is a strong predictor of early mortality, independent of MELD score. Changes in survival are especially pronounced for sodium concentrations ranging from 120 to 135 mEq/L. Within this range, a decrease in serum sodium of 1 mEq/L corresponds to a 12% decrease in 3-month probability of survival. A modified score including serum sodium, termed MELD-Na, has been proposed as an alternative to MELD score.

MELD Na = MELD – Na – (0.0025 x MELD x (140 - Na) + 140 (where the serum sodium concentration is between 125 and 140 mmol/L) (Kim WR, et

al; 2008). The accuracy of MELD-Na was shown to be slightly superior to that of MELD in candidates for transplantation. The effect of hyponatremia is higher in patients with low MELD score compared with those with high MELD score.

A limitation to the incorporation of serum sodium into MELD is that during cirrhosis, marked changes in serum sodium concentration can result from several factors, including the administration of diuretics and intravenous hypotonic fluids. For example, the administration of diuretics leads to a 4 mEq/L decrease in serum sodium, on average. In some patients the decrease may reach 10 mEq/L. In contrast, the use of V2-receptor antagonists for treating refractory ascites is encouraging. These agents induce a significant increase in serum sodium.

In patients receiving anticoagulation therapy, like Budd-Chiary syndrome and cirrhosis with portal vein thrombosis, the INR is hardly interpretable to use in the calculation of MELD score. In these cases MELD-XI (MELD excluding INR) is a good scoring method.

Patients with a rapid increase in MELD over time might be expected to have a worse outcome than those with stable or even decreasing MELD score. Delta

MELD is defined as the difference between current MELD and the lowest MELD measured within 30 days prior to current MELD, and was shown to be predictive of early mortality in patients with cirrhosis on univariate analysis (Forman LM, et al; 2001).

AIM OF THE STUDY

To study fasting serum homocysteine level in patients with alcoholic cirrhosis and viral cirrhosis and to assess the serum homocysteine level as a prognostic marker by comparing the homocysteine level with MELD score and Child-Turcotte-Pugh score.

MATERIALS AND METHODS

Place of study : Department of Digestive Health and Diseases, Government peripheral hospital, Anna nagar, Chennai.

Type of study : Prospective study.

Period of study : December 2009 to April 2011.

Collaborating Department : Department of Biochemistry, Kilpauk medical college,Kilpauk, Chennai.

Ethical committee : Approval obtained.

Consent : Informed consent obtained from all participants.

Selection of patients

Fifty patients with alcoholic liver cirrhosis and fifteen cases of viral cirrhosis who satisfied the following inclusion criteria and not having any of the exclusion criteria were selected for the study.

Inclusion criteria

1) All cases of viral markers negative alcoholic cirrhosis of liver with portal hypertension and ascites, were included in the study.
2) All cases of liver cirrhosis with portal hypertension and ascites due to chronic Hepatitis B or chronic Hepatitis C, of both sexes, without any history of alcohol intake were included in the study.

Exclusion criteria

1) Patients with both alcohol intake and viral marker positivity were excluded from the study.
2) Alcoholic hepatitis or viral chronic hepatitis patients without any clinical signs of cirrhosis were not included.
3) NASH, NAFLD were excluded.

4) Adult patients above 60 years of age were excluded to minimize the effect of old age on serum homocysteine level.
5) Patients with diabetes mellitus, chronic renal failure, hypothyroidism, inflammatory bower disease and malabsorptive conditions were excluded from the study to avoid confounding effect on homocysteine level.
6) Patients taking drugs like phenytoin, methotrexate, levodopa, fenofibrate, metformin, sulfasalazine and pyrimethamine were also excluded from the study.

Study method

Patient characteristics like age, sex and body mass index were noted. Detailed history from the patients regarding abdominal pain, abdominal distension, swelling of legs, reduced urine out put, yellowish discolouration of urine or eyes, fatigue, vomiting, vomiting of blood, passing black tarry stools, altered sleep pattern, altered sensorium, breathlessness and chestpain were taken. Any similar problem in the past and whether he is a known case of diabetes, hypertension, coronary heart disease, hypercholesterolemia, epilepsy, intestinal tuberculosis, previous jaundice, any previous abdominal surgeries

and details of the drugs he was taking so far, were asked in detail and noted. Detailed history regarding alcohol intake like brand of alcohol, quantity per day and duration of intake were asked. Similarly history regarding his smoking habits was recorded. Detailed history regarding his dietary pattern including vegetarian, non vegetarian food items and coffee intake was asked. History regarding the patients day to day physical exercise activities was also asked and recorded.

Clinical examination

Detailed general examination of the patient was done with regard to orientation to place, person and time, fever, pallor, icterus, pedal edema, clubbing, cyanosis, nutritional status, and lymphadenopathy. Vital signs were recorded for all patients. Clinical examination for cardiorespiratory status, flapping tremor and any neurological deficit were looked for. Thorough clinical examination of abdominal system was made out, particularly for the clinical signs of chronic liver disease, signs of portal hypertension, signs of hepatic encephalopathy and other complications of chronic liver disease, and clinical response to diuretics.

Investigations

Urine examination for albumin, sugar, deposits, bile salts and bile pigments was done. Complete blood count including hemoglobin, total count, differential count, platelet count and PCV were recorded. Patient's blood group was noted. Routine investigations like erythrocyte sedimentation rate, blood sugar, urea, creatinine, electrolytes - Na+, K+, Cl and HCO_3^- , were taken. Liver function tests total bilirubin, direct and indirect bilirubin, SGOT, SGPT, Alkaline Phosphatase, GGT, Total protein, Albumin, Globulin and Albumin / Globulin ratio were recorded. Prothrombin time (test & control) and INR value were noted. X-ray Chest and ECG were taken for all patients. Ultrasound abdomen was done for assessing liver size, echotexture, liver nodules, portal vein diameter, collaterals of portal hypertension, spleen size, splenic vein diameter and ascites. UGI endoscopy was done for all patients to look for esophageal or gastric varices, portal hypertensive gastropathy, gastric antral vascular ectasia, gastric or duodenal ulcers or any UGI bleed. Ascitic fluid analysis was done for protein, albumin, sugar, cell count and cytology. SAAG was calculated. Serum Homocysteine level in the morning fasting sample on the next day of admission, was estimated. Child-Turcotte-Pugh score was calculated for all patients, and using readymade calculator for

MELD score available in internet, MELD score was calculated for all patients.

Study approach

Patients with clinical signs of cirrhosis and portal hypertension of specific etiology either alcohol or viral, were selected for estimation of fasting serum homocysteine level. Fifty alcoholic cirrhosis and fifteen viral cirrhosis patients were enrolled for the study. Severity of cirrhosis was assessed for all the patients using Child-Turcotte-Pugh score and MELD score. The fasting serum homocysteine level was compared with both Child-Turcotte-Pugh and MELD scores.

RESULTS

Demography

All patients in alcoholic cirrhosis group are males (50 out of 50).

Male : Female ratio in viral cirrhosis patients is 6 : 9

Age group for alcoholic cirrhosis patients ranged from 29 to 60 years of age, and the mean age is 47.45 years.

Age group for viral cirrhosis patients ranged from 39 to 60 years of age and the mean age is 47.8 years.

Serum Homocysteine

Mean serum homocysteine level in alcoholic cirrhosis patients is 23.4 μmol/L.

Maximum value for serum homocysteine is 45.39 μmol/L and the minimum value is 12.41 μmol/L in alcoholic cirrhosis patients.

Number of alcoholic cirrhosis patients with serum homocysteine level more than the upper limit of normal value of 15 μmol/L is 41 out of 50 patients (82%).

Mean serum homocysteine level in viral cirrhosis patients is 10.05 μmol/L.

Maximum value for serum homocysteine is 15.21 μmol/L and minimum value is 7.24 μmol/L in viral cirrhosis patients.

Number of viral cirrhosis patients with serum homocysteine level more than the upper limit of normal value of 15 μmol/L is 1 out of 15 patients (6.67%).

Child – Turcotte – Pugh score

Mean CTP score for alcoholic cirrhosis patients is 8.36, maximum CTP score is 14 and minimum score is 6.

Mean CTP score for viral cirrhosis patients is 9.2, maximum CTP score is 13 and minimum score is 6.

MELD score

Mean MELD score for alcoholic cirrhosis patients is 12.22, maximum MELD score is 28 and minimum MELD score is 6.

Mean MELD score for viral cirrhosis patients is 14.93, maximum MELD score is 26 and minimum MELD score is 7.

Correlation of CTP and MELD scores

In alcoholic cirrhosis and viral cirrhosis patients, using Pearson correlation, the significance of CTP and MELD scores is calculated and the *p* value for this correlation is highly significant, $p = 0.0001$.

Correlation of serum homocysteine with CTP and MELD scores

The serum homocysteine levels correlate well with CTP and MELD scores when computed using the Pearson calculation, in both alcoholic and viral cirrhosis patients, and the *p* value for the significance is 0.0001, in both the groups.

STATISTICAL ANALYSIS

All analyses are performed using SPSS 16.0 (SPSS Inc. Chicago, IL).

Table: Descriptive Statistics

Group	**N**	**Minimum**	**Maximum**	**Mean**	**Standard Deviation**
Alcoholic group Homocysteine	50	12.41	45.39	23.3996	7.91171
CTP score	50	6	14	8.3600	2.43914
MELD score	50	6	28	12.2200	5.96004
Viral group Homocysteine	15	7.24	15.21	10.0527	2.75191
CTP score	15	6	13	9.2000	2.42605
MELD score	15	7	26	14.9333	6.40833

Independent samples test

Table: Levene's test for Equality of variances

Variable	Levene's test for Equality of variances	
	F	**Significance**
Homocysteine	10.661	0.002
CTP score	0.120	0.730
MELD score	0.467	0.497

Table: t – test for Equality of Means

Variable		t – test for Equality of Means						
		t	df	Sig 2tailed	Mean differ	Std Error differ	95% confidence Interval of the difference	
							Lower	Upper
Homocysteine	Equal variance assumed	6.388	63	0.0001	13.347	2.089	9.1717	17.522
	Equal variance not assumed	10.07	61.49	0.0001	13.347	1.325	10.697	15.997
CTP score	Equal variance assumed	-1.17	63	0.246	-0.840	0.717	-2.273	0.5932
	Equal variance not assumed	-1.17	23.17	0.252	-0.840	0.715	-2.318	0.6387
MELD score	Equal variance assumed	-1.52	63	0.133	-2.713	1.785	-6.279	0.8532
	Equal variance not assumed	-1.46	21.79	0.158	-2.713	1.857	-6.566	1.1398

Table: Correlation of serum homocysteine with CTP and MELD scores

Group	Variable	Method	CTP score	MELD score
Alcohol	Homocysteine (N=50)	Pearson Correlation	0.878*	0.845*
		Significance (*p*) (2tailed)	0.0001	0.0001
Viral	Homocysteine (N=15)	Pearson Correlation	0.903*	0.882*
		Significance (*p*) (2tailed)	0.0001	0.0001

* correlation is significant at the 0.01 level (2 tailed).

The '*p*' value for serum homocysteine level compared with CTP score and MELD scores in both alcoholic cirrhosis and viral cirrhosis patients is 0.0001.

Table: Correlation of CTP score with MELD score

Group	Variable	Method	MELD score
Alcohol	CTP score (N=50)	Pearson Correlation	0.921 *
		Significance (*p*) (2tailed)	0.0001
Viral	CTP score (N=15)	Pearson Correlation	0.966*
		Significance (*p*) (2tailed)	0.0001

* correlation is significant at the 0.01 level (2 tailed).

The '*p*' value for CTP score compared with MELD score in both alcoholic cirrhosis and viral cirrhosis patients is 0.0001.

Hence, the serum homocysteine level highly correlates with both CTP score and MELD score in both alcoholic cirrhosis and viral cirrhosis patients and hence indicates the severity of liver cirrhosis.

DISCUSSION

Studies in cultured hepatocytes suggest a role of the liver in metabolism of homocysteine. In fact, hyperhomocysteinaemia has been reported in several experimental models of liver damage (Garcia-Tevijano ER, Ferre N, et al; 2002). Chronic treatment with ethanol or CCl4 in experimental animals is associated with hyperho-mocysteinaemia, and the hepatoprotective effect of S-adenosyl-methionine on experimental cirrhosis is accompanied by a normalization of methionine metabolism and a decrease in homocysteine concentration (Ferre N, et al; 2002). However, the presence and degree of hyperhomocysteinaemia in patients with liver disease is, as yet, not well defined.

In a study at King George's medical university, Lucknow, India, in 2005, comprising of 30 patients with cirrhosis of liver, including 12 alcoholic and 18 non alcoholic cirrhosis, serum homocysteine levels were found to be elevated in 68% cases. The serum homocysteine levels were significantly elevated in chronic liver disease cases compared to controls, to 13.44 $\pm$ 8.09 μmol/L ($p < 0.001$). The levels were significantly elevated in alcoholic cirrhosis patients to 23.04 $\pm$ 10.67 μmol/L, compared to non alcoholics (10.78 $\pm$ 4.22 μmol/L), p value < 0.001.

In Portugal study, the serum homocysteine levels in chronic alcoholics were found to be 21.2 $\pm$ 8.0 μmol/L, twice as high as controls with a *p* value of 0.05 (Marilia L Cravo, et al; 1996).

In another study at England, hyperhomocysteinemia (concentration > 12 mmol//l) was stated in 70% of the alcoholic cirrhosis patients and mean homocysteine concentration was statistically significantly higher if compared to the controls (13.29 $\pm$ 8.16 vs 8.03 $\pm$ 1.6 μmol/L, $p < 0.05$) (Kazimierska E, et al; 2003).

In one study at Slovak republic, a statistically significant increase of serum homocysteine was seen in all groups of patients with chronic liver diseases: steatosis 12.1 $\pm$ 7.3, ($p < 0.01$), mild fibrosis/cirrhosis, 14.1 $\pm$ 10.8, ($p < 0.01$), up to severe cirrhosis, 16.9 $\pm$ 10.9, ($p < 0.001$), (Anna Remkova, Milan Remko, et al; 2009).

Forty three biopsy proven cirrhosis patients of different etiology were studied at France in 2001 and the results were: 74% of the patients had elevated plasma homocysteine levels defined as >13.4 μmol/L (mean+2SD of healthy age matched controls). Increased plasma homocysteine concentrations were

seen in alcoholic as well as in non-alcoholic cirrhotics (Anja Bosy-Westphal, et al; 2001). But in the present study, serum homocysteine levels were significantly elevated in alcoholic cirrhosis patients (82%) than in non alcoholic viral cirrhosis patients (6.67%).

In a study at United States of America, including 40 alcoholic cirrhosis patients, 26 active alcohol drinkers without clinical evidence of liver disease and 28 healthy controls, the median homocysteine level in alcoholic cirrhosis patients was 10.2 μmol/L, with a range of 5.4 to 58.3 μmol/L. The median homocysteine level in active alcohol drinkers was 8.8 μmol/L, with a range of 5.8 to 23 μmol/L, and in the healthy controls the median value was 6.4 μmol/L, with a range of 4.1 to 10 μmol/L, in the same study. The *p* value for increase in homocysteine level was < 0.0001 in both alcoholic cirrhosis and active alcohol drinkers when compared with healthy controls (Valentina Medici, et al; 2010). In the same study, the elevation in homocysteine level when compared with CTP and MELD scores, the *p* values were 0.041 and 0.02 respectively.

In the present study, serum homocysteine levels are elevated more than the upper limit of normal (15 μmol/L) in 41 out of 50 (82%) alcoholic cirrhosis

patients compared to only one patient out of 15 (6.67%) viral cirrhosis patients. The average serum homocysteine level in alcoholic group is 23.4 $\pm$ 7.91 μmol/L compared to 10.05 $\pm$ 2.75 μmol/L in viral cirrhosis group. The '*p*' value for variation in serum homocysteine level corresponding to CTP and MELD scores is < 0.0001 in both alcoholic and viral cirrhosis patients. But the '*p*' value can be taken as significant, only in alcoholic cirrhosis patients, as there is only one patient with elevated homocysteine level in the viral cirrhosis group.

CONCLUSION

1) Serum homocysteine levels are significantly elevated in alcoholic cirrhosis patients (82%).
2) Serum homocysteine levels are not significantly elevated in viral cirrhosis patients (6.67%).
3) Serum homocysteine levels correlated significantly with CTP and MELD scores in both alcoholic cirrhosis and viral cirrhosis patients.
4) Fasting serum homocysteine level may be used as a prognostic marker in alcoholic cirrhosis patients.

APPENDIX B

MASTER CHART FOR ALCOHOLIC CIRRHOSIS PATIENTS

S.No	Name of the patient	Age/ Sex	Serum Bilirubin	Serum Albumin	Serum Creatinine	INR	Ascites	Encephalopathy	CTP score	MELD score	Serum Homocysteine
1	Mohammed	48/M	2.1	3.3	0.8	1.54	Mild	Nil	8	12	30.08
2	Parthiban	38/M	17.6	2.2	1.1	2.01	Severe	Gr3	14	26	27.91
3	Devaraj	50/M	2.3	3.4	1.0	1.48	Mild	Nil	8	14	14.97
4	Suresh	36/M	17.8	3.4	1.2	2.14	Severe	Gr3	13	28	45.39
5	Kannappan	55/M	1.4	3.7	0.8	1.23	Mild	Nil	6	8	14.65
6	Kumaresan	49/M	7.8	2.5	1.2	1.58	Severe	Gr3	13	21	32.43
7	Kuppan	44/M	3.2	2.7	1.2	1.48	Mild	Nil	10	17	29.31
8	Rajagopal	56/M	11.7	3.3	1.0	1.33	Mild	Nil	9	19	28.32
9	Panneerselvam	54/M	4.3	3.6	0.9	1.26	Mild	Nil	8	14	27.61
10	Ramkumar	39/M	2.3	3.7	0.8	1.23	Mild	Nil	7	10	23.56
11	Munusamy	43/M	3.4	3.2	0.9	1.28	Mild	Gr1	10	13	27.10
12	Kumarimuthu	29/M	0.8	3.9	0.6	1.20	Mild	Nil	6	6	16.92
13	Anandhan	33/M	2.7	3.4	0.8	1.26	Mild	Nil	8	11	19.73
14	Venkatesan	45/M	4.2	2.9	0.9	1.54	Severe	Gr1	11	16	33.57
15	Gurujikannappan	60/M	5.4	2.7	1.3	1.64	Severe	Gr1	12	21	38.12
16	Rajkumar	49/M	2.2	3.2	0.7	1.26	Mild	Nil	8	9	22.83
17	Balan	56/M	1.2	3.7	0.8	1.30	Mild	Nil	6	8	14.24
18	Govindhan	50/M	0.9	3.3	0.7	1.17	Mild	Nil	7	6	18.47
19	Ramadoss	42/M	1.3	2.6	0.8	1.08	Mild	Nil	8	6	17.40
20	Devadoss	47/M	0.9	2.7	0.8	1.13	Mild	Nil	8	6	20.83
21	Jayakumar	55/M	1.1	3.1	0.7	1.30	Mild	Nil	7	6	22.61
22	Sivakumar	50/M	2.2	3.4	1.0	1.43	Mild	Gr1	9	13	20.32
23	Ramanathan	40/M	1.4	3.7	0.7	1.32	Mild	Nil	6	7	16.94
24	Saravanan	38/M	5.9	2.5	1.3	1.83	Severe	Gr3	14	22	42.16
25	Kumar	42/M	3.8	3.1	1.1	1.50	Mild	Gr1	10	17	31.18

S.No	Name of the patient	Age/ Sex	Serum Bilirubin	Serum Albumin	Serum Creatinine	INR	Ascites	Encephalopathy	CTP score	MELD score	Serum Homocysteine
26	Kanniappan	43/M	1.5	2.2	0.9	1.41	Mild	Nil	8	11	18.71
27	Vijayakumar	38/M	0.6	3.6	0.6	1.10	Mild	Nil	6	6	12.41
28	Pachiappan	60/M	0.8	3.7	0.9	1.16	Mild	Nil	6	6	13.90
29	Raja	55/M	1.8	3.2	0.9	1.31	Mild	Nil	7	11	18.12
30	Kuppan	44/M	3.2	2.7	1.2	1.46	Mild	Nil	10	17	29.31
31	Rajagopal	56/M	1.7	3.3	1.0	1.32	Mild	Nil	7	12	18.32
32	Panneerselvam	54/M	1.3	3.6	0.9	1.26	Mild	Nil	6	9	17.61
33	Kumaresan	49/M	7.8	2.5	1.2	1.71	Severe	Gr2	13	22	32.43
34	Vellaiappan	45/M	1.2	3.5	0.9	1.23	Mild	Nil	7	8	19.22
35	Muthukumar	39/M	1.4	3.7	0.8	1.35	Mild	Nil	6	9	14.57
36	Gopinath	44/M	3.9	3.1	1.2	1.49	Mild	Gr1	10	18	25.31
37	Raghuram	54/M	1.8	3.5	1.0	1.26	Mild	Nil	7	11	22.10
38	Sathyaseelan	45/M	1.3	3.7	0.8	1.24	Mild	Nil	6	8	14.57
39	Kandhasamy	40/M	0.9	3.6	0.7	1.16	Mild	Nil	6	6	13.28
40	Kumaravel	58/M	1.1	3.4	0.8	1.20	Mild	Nil	7	7	19.57
41	Anandhm	60/M	7.7	2.5	1.4	1.76	Severe	Gr1	13	24	37.18
42	Thirumaran	52/M	1.3	3.6	0.9	1.10	Mild	Nil	6	7	14.75
43	Arokiyasamy	60/M	2.4	3.2	0.9	1.17	Mild	Nil	8	10	21.33
44	Selvam	47/M	6.6	2.7	1.3	1.35	Severe	Gr1	12	19	36.84
45	Anbuselvan	39/M	1.4	3.8	0.7	1.13	Mild	Nil	6	6	21.13
46	Kumarimuthu	55/M	2.4	3.3	0.8	1.16	Mild	Nil	8	9	24.58
47	Sundaram	52/M	1.5	3.5	0.7	1.22	Mild	Nil	7	7	23.12
48	Loganathan	47/M	1.3	3.6	0.9	1.31	Mild	Nil	6	9	19.87
49	Savarimuthu	41/M	2.7	3.6	1.0	1.23	Mild	Nil	7	13	20.94
50	Balakumar	53/M	1.9	3.2	0.8	1.30	Mild	Nil	7	10	24.16

MASTER CHART FOR VIRAL CIRRHOSIS PATIENTS

S.No	Name of the patient	Age/ Sex	Serum Bilirubin	Serum Albumin	Serum Creatinine	INR	Ascites	Encephalopathy	CTP score	MELD score	Serum Homocysteine
1	Amaresan	41/M	1.8	2.7	0.9	1.52	Mild	Nil	8	9	9.23
2	Karthikeyan	44/M	13.6	2.2	1.3	1.91	Severe	Gr1	13	26	13.41
3	Jeyarani	56/F	2.5	3.4	0.9	1.48	Mild	Nil	8	13	7.97
4	Surendran	39/M	7.8	3.3	1.2	1.98	Severe	Gr1	12	24	15.21
5	Kanniammal	54/F	1.3	3.6	0.8	1.23	Mild	Nil	6	8	8.45
6	Vijayalaxmi	39/F	7.9	2.5	1.3	1.54	Severe	Gr1	12	22	11.68
7	Kuppammal	44/F	3.3	2.7	1.1	1.45	Mild	Nil	10	16	9.35
8	Rajaguru	55/M	6.7	3.3	1.0	1.26	Mild	Nil	9	16	8.42
9	Anandhavalli	52/F	3.7	3.7	0.8	1.30	Mild	Nil	8	12	7.46
10	Ravikumar	47/M	2.6	3.6	0.8	1.17	Mild	Nil	7	10	8.35
11	Venkatesh	41/M	7.2	2.6	1.4	1.54	Severe	Gr1	12	22	13.56
12	Sivakami	60/F	4.4	2.7	1.3	1.64	Severe	Gr1	12	20	14.15
13	Rajakumari	45/F	2.4	3.1	0.8	1.26	Mild	Nil	8	10	8.84
14	Bakyam	50/F	1.3	3.6	0.9	1.30	Mild	Nil	6	9	7.24
15	Govindhammal	50/F	1.4	3.3	0.8	1.17	Mild	Nil	7	7	7.47

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APPENDIX A

PROFORMA

Name of the patient : S.No :

Age / Sex :

IP No :

DDHD No :

Presenting symptoms : Abdominal pain, abdominal distension, swelling of legs, reduced urine out put, yellowish discolouration of urine or eyes, fatigue, vomiting, vomiting of blood, passing black tarry stools, altered sleep pattern, altered sensorium, breathlessness,

Past H / O similar problem before

Past H / O : DM, HT, TB, COPD, CAHD, Chronic drug intake, Abdominal surgery, Jaundice

Personal history : Diet, Smoking, Alcohol (brand, quantity, duration)

General examination : BMI: Orientation / Fever / Pallor / Icterus / Pedal edema / Clubbing / Cyanosis / Lymphadenopathy / Others

Vital signs : Pulse rate: BP: Resp.rate: Temp:

Abdomen :

Signs of CLD :

CVS :

RS :

CNS :

Investigations

Urine : Alb - Sugar - Dep - BS - BP –

CBC : Hb - TC - DC - Platelet - PCV –

ESR : Blood group:

Blood : Sugar - Urea - Creatinine –

Electrolytes : Na^+ - K^+ - Cl^- HCO_3^- -

LFT : Total bilirubin - Direct - Indirect –

SGOT - SGPT - Alk.Phos - GGT –

Total protein - Albumin - Globulin –

Prothrombin time : Test - Control - INR -

X-Ray Chest :

ECG :

USG abdomen :

UGI endoscopy :

Ascitic fluid : Protein - Albumin - Sugar - Cell count –

Cytology - Others -

SAAG :

Serum Homocysteine :

MELD score :

Child-Turcotte-Pugh score :